Irradiation for Quarantine Control of the Invasive Light Brown Apple Moth (Lepidoptera: Tortricidae) and a Generic Dose for Tortricid Eggs and Larvae

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ABSTRACT The effects of irradiation on egg, larval, and pupal development, and adult reproduction in light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), were examined. Eggs, neonates, third instars, fifth instars, and early stage pupae were irradiated at target doses of 60, 90, 120, or 150 Gy or left untreated as controls in replicated factorial experiments and survival to the adult stage was recorded. Tolerance to radiation generally increased with increasing age and developmental stage. A radiation dose of 120 Gy applied to eggs and neonates prevented adult emergence. A dose of 150 Gy prevented adult emergence in larvae at all stages. In large-scale validation tests, a radiation dose of 150 Gy applied to fifth instars in diet, apples or peppers resulted in no survival to the adult stage in 37,947 treated individuals. Pupae were more radio tolerant than larvae, and late stage pupae were more tolerant than early stage pupae. Radiation treatment of late pupae at 350 and 400 Gy resulted in three and one fertile eggs in 4,962 and 4,205 total eggs laid by 148 and 289 mating pairs, respectively. For most commodities, the fifth instar is the most radio tolerant life stage likely to occur with the commodity; a minimum radiation dose of 150 Gy will prevent adult emergence from this stage and meets the zero tolerance requirement for market access. For traded commodities such as table grapes that may contain *E. postvittana* pupae, a radiation dose >400 Gy may be necessary to completely sterilize emerging adults. After review of the literature, a generic radiation treatment of 250 Gy is proposed for tortricid eggs and larvae in regulated commodities.

KEY WORDS x-ray radiation, Lepidoptera, invasive species, regulatory pest, phytosanitary treatment

Light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) is a native pest of Australia and is now widely distributed in New Zealand, New Caledonia, Hawaii, the United Kingdom, and Ireland. *E. postvittana* is highly polyphagous and a serious economic pest to a wide range of horticultural crops (Suckling and Brockerhoff 2010). In March 2007, *E. postvittana* was detected for the first time in several counties in California, triggering the issuance of a federal domestic quarantine order. The United States Department of Agriculture (USDA) quarantine order restricts the intra- and interstate movement of plant materials, and requires regulated articles to be visually inspected and certified as free of *E. postvittana* life stages.

A major concern with *E. postvittana* establishment and spread in California is trade restrictions on fruits and vegetables imposed by importing countries. This moth is an important economic pest of fruits such as apples, apricots, citrus, grapes, nectarines, peaches, plums, pears, and sweet cherries, and can be found on forestry, vegetable and nursery crops (Wearing et al. 1991). *E. postvittana* is currently on the list of harmful organisms in 11 countries including Canada, Chile, Ecuador, India, Japan, South Korea, Mexico, Peru, South Africa, Taiwan, and Thailand. Canada, Chile, India, Mexico, and South Africa require phytosanitary certificates ensuring that products have been inspected and found free of any stage of *E. postvittana* (U.S. Department of Agriculture–Animal and Plant Health Inspection Service [USDA–APHIS] 2012). Mexico and Canada have imposed restrictions on the importation of crops and plants from infested areas in California and Hawaii (Varela et al. 2008).

E. postvittana damage is typically to the foliage as larvae roll the leaves and feed on leaf edges, but may extend to the fruit surface if leaves contact the fruit or when two fruit are close together (Wearing et al. 1991, Varela et al. 2008, Suckling and Brockerhoff 2010). Rarely young larvae may feed internally in the calyx region of pome and stone fruits where they would be difficult to detect during packinghouse sorting and grading (Whiting et al. 1997). The incidence of surface feeding by *E. postvittana* during inspection of New

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Zealand apples exported to the United States is ≈0.1%, whereas the estimated frequency of larvae inside apple calyxes is estimated at 1×10^{-6} (J. Walker, personal communication). Grapes can be severely damaged by larvae feeding among the berries, and injured fruit become infected with mold (Lo and Murrell 2000).

Irradiation is a postharvest quarantine treatment option for exported commodities such as fruits and vegetables to prevent movement of viable *E. postvittana.* Irradiation treatment is effective against insect pests at doses that typically do not harm the quality of fresh commodities (Wall 2008, Follett 2009). In 2006, USDA–APHIS approved generic radiation doses of 150 Gy for any tephritid fruit fly and 400 Gy for all other insects except the pupa and adult stages of Lepidoptera (that may require higher doses) (USDA–APHIS 2006). During commercial irradiation, the actual dose received by the commodity varies because the ionizing radiation must penetrate to the center of a three-dimensional load of boxes (Follett and Weinert 2009). The dose-uniformity ratio (DUR = maximum/minimum dose) for commercial irradiators can range from 1.5 to 3.0 (Follett 2006a, Mehta and O'Hara 2006). Applying a radiation dose of 400 Gy to a commodity against *E. postvittana* life stages, therefore, may result in part of the load receiving doses between 600–1,200 Gy. Irradiation treatment at this level may negatively affect commodity quality (Wall 2008), and the treatment may exceed the limit of 1,000 Gy (1 kGy) established by the Food and Drug Administration (FDA) for fresh commodities. Lowering the dose from 400 Gy for *E. postvittana* would reduce any deleterious effects on quality, ensure the applied treatment dose is below the one kGy limit, and lower the cost of treatment owing to shorter treatment time (Follett 2010).

The objective of the study with *E. postvittana* reported here was to examine the effects of irradiation on egg, larval, and pupal development, and adult reproduction, and to conduct validation tests with a radiation dose predicted to control *E. postvittana* to demonstrate it provides quarantine security.

Materials and Methods

Insect Rearing. A laboratory colony of *E. postvittana* was started using larvae (≈100) collected mainly from *Lotus pedunculatus* Cav. leaves from Volcano, HI. Larvae were removed from rolled leaves and placed directly on pink bollworm diet (Stewart 1984). The pink bollworm diet used throughout the study was obtained from the USDA-APHIS Pink Bollworm Rearing Facility in Phoenix, AZ (Miller et al. 1996). Pupae were transferred to petri dishes in the bottom of screen cages for moth emergence. When adults began to emerge and mate, plastic cup lids (8 cm diameter; Solo Cup Company, Chicago, IL) were hung from three interior sides of the cages as an oviposition substrate. Female moths began laying eggs within 24 h and completed laying eggs within 3 d. Larvae were reared in 830 ml plastic food service tray with clear lids (TriPak Industrial Corp., Toronto, Ontario, Canada). A section of light diffuser panel was cut to size and inserted in the bottom of each plastic food service tray (Clare et al. 1987), and then diet was packed into the panel grid. A second section of diffuser panel was placed in the container on top of the first panel. Cup lids with eggs were placed on top of the second panel until eggs had hatched and neonates had moved down into the diet. A 20 × 20 cm piece of wax paper (Wax-Tex Inc., Menominee Paper Co., Menominee, MI) was folded accordion-style and placed on top of the light diffuser panel as a pupation site. Rearing conditions were 24°C (±2°C), ≈70% relative humidity (RH), and a photoperiod of 12:12 (L:D) h for the duration of the experiments. Larvae are easily removed from plastic food service trays by pulling the panels apart (larval webbing holds the panels together) and using soft forceps. Pupae can be removed likewise from the wax paper.

Irradiation Treatment. Irradiation treatment was conducted at a nearby commercial x-ray facility (CW Hawaii Pride LLC, Keaau, HI) using an electron linear accelerator (5 MeV, model TB-5/15, L-3 Communications Titan Corp., San Diego, CA) at ambient temperature (19°C). Dosimeters (Opti-chromic detectors, FWT-70–83M, Far West Technology, Goleta, CA) were placed in between containers containing eggs, larvae, or pupae at each dose in each replicate. The dosimeters were read with a FWT-200 reader (Far West Technology) at 600-nm absorbance to measure dose variation in each treatment replicate. To minimize the dose uniformity ratio (the ratio of the maximum/minimum dose), plastic containers with leafrollers were placed in a single row perpendicular to the x-ray beam and elevated by placement on a cardboard box positioned in the center of the carrier. Each carrier passed in front of the beam in a forward then reverse orientation. The carriers complete the circuit through the radiation chamber in 15 min and the actual time in front of the electron beam/x-rays for each plastic container with insects is 30 s. The dose uniformity ratio during the *E. postvittana* research was generally <1.2. After irradiation treatment, *E. postvittana* life stages were returned to the laboratory and held under standard rearing conditions.

Experimental Design. Eggs (6- to 7-d-old [normally eggs hatch after 8 d at 24°C]) in plastic containers without diet, and larvae (first, third, and fifth instars) on diet, were irradiated at target doses of 60, 90, 120, and 150 Gy in replicated factorial experiments. Early (1- to 3-d-old) and late (8- to 10-d-old) stage pupae also were tested but at 150, 200, 250, 300, 350, and 400 Gy. Pupal development to the adult stage is 8–11 d under our rearing conditions (24 ± 2°C). Larvae were tested in 473-ml plastic cups with 100 g diet, whereas eggs attached to plastic lids and pupae were irradiated in 473-ml plastic cups without diet. After treatment, eggs and larvae were followed until pupation and adult emergence. Irradiated early (1-to 3-d old) and late (8- to 10-d old) pupae were sexed and transferred to 473-ml plastic cups for adult emergence. Eight females and 10 males were placed in each mating cup with a

Table 1. Maturation of *Epiphyas postvittana* eggs, larvae, and early pupae after irradiation

Stage	Dose (Gy)	Reps	No. treated	No. pupae	No. adults (±SE)	% pupated (±SE)	% adult emergence
Egg	0	5	500	154	123	30.8 (5.7)	24.6 (4.2)
	30	5	500	48	26	9.6 (2.4)	5.2 (1.8)
	60	5	500	47	14	9.4 (3.5)	2.8 (1.2)
	90	5	500	24	2	4.8 (3.1)	0.4 (0.2)
	120	5	500	12	0	2.4 (1.2)	0.0
	150	5	500	0	0	0.0	0.0
L1	0	7	255	188	151	75.4 (6.3)	61.0 (6.5)
	60	7	255	1	13	29.5 (8.1)	6.2 (2.7)
	90	7	255	27	2	10.3 (1.3)	1.0 (0.6)
	120	7	255	13	0	5.2 (0.7)	0.0
	150	7	255	0	0	0.0	0.0
L3	0	6	260	212	190	81.6 (3.3)	72.1 (3.9)
	60	6	260	183	81	71.6 (2.6)	33.0 (2.2)
	90	6	275	149	32	60.5 (8.8)	13.2 (3.5)
	120	6	275	107	3	46.9 (10.5)	1.4 (1.1)
	150	6	275	72	0	33.2 (9.3)	0.0
L5	0	6	240	200	176	84.2 (2.9)	75.5 (4.5)
	60	6	240	178	50	77.6 (5.0)	27.5 (8.1)
	90	6	255	115	14	47.7 (5.8)	7.5 (3.6)
	120	6	255	106	4	44.5 (5.2)	2.2 (1.6)
	150	6	255	91	0	40.5 (7.3)	0.0
P1	0	3	127	—	117	—	92.2 (2.8)
	60	3	144	—	122	—	84.9 (4.3)
	90	3	132	—	98	—	73.8 (4.5)
	120	3	112	—	66	—	60.0 (10.6)
	150	3	125	—	84	—	66.7 (7.2)

Stages: eggs; L1, neonates; L3, third instars; L5, fifth instars; P1, early pupae (1- to 3-d old).

water source and followed for 2 wk or until all individuals had died. Adult emergence and the number of eggs laid (fecundity) and eggs hatched (fertility) was recorded. For each larval or pupal age/stage a minimum of four replicates were irradiated, and in each replicate a control group of the same life stage was not irradiated and held under the same conditions.

A test was conducted to compare the response to irradiation in larvae treated in artificial diet with those treated in fruit. Fifth instars were counted and placed on diet or artificially inoculated into bell peppers, *Capsicum annuum* (20 per fruit) for irradiation treatment. For treatment in peppers, two holes were made with a 12 mm core punch on opposite sides of the fruit and 10 insects were placed inside the cavity before taping the fruit closed. Fruit or diet with insects were irradiated at sublethal doses of 50 or 100 Gy or left untreated as a control. Each treatment had eight replicates with 20 individuals and all replicates were treated on the same day. Dosimeters were placed inside representative fruit and food service trays with diet in each replicate to measure dose variation. After treatment, larvae treated in fruit were placed back on artificial diet to improve survivorship. Larvae from the artificial diet and fruit treatments were followed until pupation and adult emergence. Only adult emergence data are presented.

E. postvittana eggs and larvae are the life stages likely to occur with exported fresh fruits and vegetables, and these life stages occur on the surface rather than inside of the commodity. Large-scale validation testing was done with fifth instars (the most radiation tolerant stage) at a radiation dose of 150 Gy to confirm the efficacy of this dose as a potential quarantine treatment. The desired response for an effective irradiation treatment against larvae is to prevent adult emergence, and therefore methods were nearly identical to those described above for the larval dose–response tests. Fifth instars were counted and placed on diet or artificially inoculated into apples, *Malus domestica* (10 per fruit) or bell peppers (20 per fruit) for irradiation treatment. For treatment in apples and peppers, fruit were cut open and a cavity was hollowed out before placing insects inside and taping the fruit closed. Dosimeters were placed inside representative fruit and food service trays with diet in each replicate to measure dose variation. The majority of fifth instars were irradiated in diet to facilitate treatment of a large number of individuals, and because feeding internally in fruit is a rare event and more common in early than late instars when it does occur (Whiting et al. 1997; J. Walker, personal communication).

Statistical Analysis. To make comparisons of radiation tolerance between life stages, dose–response data on percent survival to the adult stage were arcsine transformed and subjected to linear regression and analysis of covariance (ANCOVA) using the standard least squares model (SAS Institute 2002). Data used in the regression model and covariance analysis included any radiation dose causing mortality between 0 and 100%, and the lowest dose causing 100% mortality. For each replicate, mortality values <100% were adjusted for control mortality using Abbott's formula (Abbott 1925). Residual plots were evaluated to ensure regression model assumptions were met for each treatment combination. Covariance analysis requires the slopes of the regression lines fitted to each group to be parallel, so the assumption of parallelism (nonsignificant life stage × dose interaction effect) was tested before

Table 2. Linear regressions on prevention of development to adult when various life stages of *Epiphyas postvittana* were irradiated at 60, 90, 120, and 150 Gy

Stage	Obs.	Y-intercept (±SE)	Slope (±SE)	R^2	Predicted doses (±95% CL)	
					LD_{90}	$LD_{99.9968}$
Egg	5	73.4 ± 5.4	0.24 ± 0.07	0.42	69.8 (43.0–91.5)	111.9 (90.5–170.5)
L1	7	83.7 ± 4.6	0.14 ± 0.05	0.31	43.5 (−81.1–67.5)	112.8 (94.8–180.3)
L3	6	28.1 ± 7.2	0.52 ± 0.07	0.75	118.3 (109.6–129.0)	137.5 (127.1–152.5)
L5	6	48.0 ± 10.0	0.38 ± 0.10	0.45	111.1 (93.4–131.5)	136.4 (118.6–174.4)
Pupae (early)	3	4.7 ± 11.8	0.23 ± 0.11	0.31	374.7	418.7

Regression analysis used adult survivorship data from Table 1 converted to percentage mortality and adjusted for control mortality using Abbott's formula.

evaluating intercepts (life stage effects) (Sokal and Rohlf 1981).

Data on percentage egg hatch in irradiated early and late stage pupae also was subjected to regression and covariance analysis to determine the effects of treatment on adult sterility. Data on percentage adult emergence in fifth instars irradiated in peppers and diet was subjected to analysis of variance (ANOVA) before and after adjusting for control mortality, and means separations were done for each dose separately using a *t*-test at the 0.05% level of probability. For large scale confirmatory tests, the level of confidence associated with treating a number of insects with zero survivors is given by the equation,

$$C = 1 - (1 - p_u)_n$$

where p_u is the acceptable level of survivorship (as a proportion) and n is the number of test insects (Couey and Chew 1986). Confidence levels were calculated for the number of treated *E. postvittana* fifth instars assuming the required efficacy ($[1 - p_u] \times 100$) is 99.99%.

Results

In general, tolerance to radiation in *E. postvittana* increased with increasing age and developmental stage (Table 1). No eggs or first instars developed to the adult stage at a radiation dose of 120 Gy, and no third or fifth instars developed to the adult stage at a radiation dose 150 Gy. Early pupae readily completed development and emerged as adults in all radiation treatments. Covariance analysis of dose–response data were significant ($P < 0.01$) for the effect of life stage, irradiation dose, and the life stage by irradiation dose interaction (Table 1). For development to the adult stage, eggs were less radio tolerant than third instars (*t*-ratio = 6.2; $P < 0.001$), fifth instars (*t*-ratio = 6.2; $P < 0.001$), and early pupae (*t*-ratio = 14.3; $P < 0.001$); first instars were less radio tolerant than third instars (*t*-ratio = 7.3; $P < 0.001$), fifth instars (*t*-ratio = 4.0; $P < 0.001$), and early pupae (*t*-ratio = 18.7; $P < 0.001$); and third and fifth instars were less radio tolerant than early pupae (*t*-ratio = 14.4, $P < 0.001$; and *t*-ratio = 11.9, $P < 0.001$, respectively). Radio tolerance of eggs and first instars was not significantly different (*t*-ratio = −0.87; $P = 0.39$), nor was that of third and fifth instars (*t*-ratio = −1.5; $P = 0.13$).

The data shown in Table 1 were converted to percentage mortality and subjected to linear regression analysis. Linear regression was used to test whether slopes were significantly different from 0 (significant effect of radiation dose), and to predict a radiation dose for larvae needed to prevent adult emergence in *E. postvittana.* Slopes were positive and significant for all life stages ($P < 0.001$), indicating that mortality increased with increasing dose (Table 2). Third instars and fifth instars were nearly identical in their response to irradiation. Typically, radiation tolerance increases with age (Dentener et al. 1990, Follett and Lower 2000, Hollingsworth and Follett 2007, Follett 2008), so the fifth instar was selected for further testing to identify a potential quarantine treatment. The desired response for radiation treatment of fifth instars is prevention of adult emergence, that is, mortality before reaching the adult stage. The linear regression equation describing data for fifth instar mortality was y (percent mortality) = 48.0–0.38 (dose) ($R^2 = 0.45$) (Table 2). The predicted dose to prevent development to adult was 136 Gy with a 95% CI of 118–174 Gy.

In the study comparing radiation tolerance in diet and fruit, survivorship to the adult stage was significantly higher for fifth instars in diet compared with pepper in the untreated controls ($P < 0.01$) and at 50 Gy ($P < 0.05$), but similar at 100 Gy ($P = 0.31$; Table 3). After adjusting for control mortality, survivorship to the adult stage was not significantly different between diet and pepper at 50 or 100 Gy, although the trend was for higher survivorship on diet after irradiation (Table 3). This indicates that treatment of fifth instars on diet will provide sufficient data for devel-

Table 3. Survivorship to adult in fifth instar *Epiphyas postvittana* irradiated in artificial diet or pepper

Substrate	Adult emergence (mean % ± SE)[a]		
	Control (0 Gy)	50 Gy	100 Gy
Diet	81.0 (4.8)a	72.0 (6.9)a	11.0 (2.9)a
Pepper	62.2 (3.6)b	53.0 (4.9)b	7.0 (2.0)a
Diet (adjusted)[b]	—	87.9 (15.6)a	10.1 (4.5)a
Pepper (adjusted)[b]	—	79.7 (15.9)a	8.7 (2.7)a

[a] Means within a column followed by different letters for the diet vs pepper and diet (adjusted) vs pepper (adjusted) comparisons are significantly different using a *t*-test ($P < 0.05$).

[b] Survivorship adjusted for control mortality using Abbott's formula.

Table 4. Large-scale validation tests to prevent adult emergence from irradiated *Epiphyas postvittana* fifth instars

Substrate	Target dose (Gy)	Measured doses	No. replicates[a]	No. treated	No. pupae	No. adults
Diet	150	134–149	33	34,997	17,470	0
	Control	0	33	2,966	2,597	2,163
Apple	150	139–150	5	2,650	725	0
	Control	0	5	330	214	170
Pepper	150	135–145	1	300	77	0
	Control	0	1	75	54	48

[a] Treatment dates served as replicates.

opment of a quarantine radiation treatment for fruit. In large-scale validation tests, a radiation dose of 150 Gy (measured doses 134–150 Gy) applied to fifth instars in diet or apples or peppers resulted in no survival to the adult stage in 37,947 treated individuals (Table 4). Assuming a required efficacy of 99.99%, $C = 1 - (1-0.0001)^{37,947}$ and our confidence level was 97.8% that the true survival of *E. postvittana* was <0.0001.

In certain crops such as grapes the pupal stage of *E. postvittana* may be found with the exported commodity. A radiation treatment causing adult sterility may be required in such cases to provide quarantine security. Late pupae were more radio tolerant than early pupae (t-ratio = 4.5; $P < 0.001$) in terms of the percentage of eggs hatching from mated female moths (Table 5). Regression analysis on the percentage of eggs hatched can be used to estimate a dose for pupae sufficient to sterilize adults (Table 6). The linear regression equation describing the data for late pupae on egg hatch was y (percentage egg hatch) = 32.7–0.097 (dose) ($R^2 = 0.64$). The predicted dose to prevent egg hatch was 337 Gy with a 95% CI of 322–354 Gy (Table 6); however, radiation treatment of late pupae at 350 and 400 Gy during dose–response testing resulted in three and one fertile eggs out of 4,962 and 4,205 eggs laid by 148 and 289 mating pairs, respectively (Table 5). Therefore, a radiation dose of 350 and even 400 Gy applied to late pupae did not cause complete sterility in adults.

Discussion

In general, tolerance to radiation in *E. postvittana* increased with increasing age and developmental stage. A radiation dose of 150 Gy applied to fifth instars prevented maturation to the adult stage in 37,947 treated individuals. In most cases, the fifth instar is the most advanced and radio tolerant *E. postvittana* life stage that may be present in fruit. A radiation dose of 150 Gy is recommended to control any *E. postvittana* eggs and larvae present in regulated fruits and vegetables. The late pupa was the most radio tolerant stage tested. For commodities such as table grapes that may contain *E. postvittana* pupae, a radiation dose ≥400 Gy may be required for quarantine control when the required response is adult sterility. Large-scale validation testing with late pupae is needed to confirm a treatment sufficient to provide the desired level of quarantine security.

After tephritid fruit flies, tortricid moths are probably the most significant pests of economic and quarantine concern for fruits (Bloem et al. 2003). USDA–APHIS has expressed interest in developing additional generic doses for broad groups of quarantine pests, particularly for the Tortricidae. Phytosanitary irradiation has been studied in ≈10 species of tortricids of economic and quarantine importance (Table 7). The number of insects tested was relatively low in several studies and not sufficient to predict with high certainty a dose that would provide quarantine security (Follett and Neven 2006). Before recommending a generic treatment for this group, a margin of safety should be added to the dose controlling the most tolerant species.

Oriental fruit moth, *Grapholita molesta* (Busck), may be one of the most radio tolerant tortricid larvae studied thus far. In ambient atmospheres, 110 out of 18,306 fifth instars irradiated at a target dose of 175 Gy emerged as adults. No adults emerged from 58,779 fifth instars irradiated at a target dose of 200 Gy (195–232 Gy measured) (Hallman 2004). In atmospheres flushed with nitrogen, however, 5.3% of oriental fruit moth adults emerged from 44,050 fifth instars irradiated with a target dose of 200 Gy (194–230 Gy measured), but laid no eggs, indicating that radio tolerance

Table 5. Adult reproduction after irradiation of *Epiphyas postvittana* pupae

Pupal age	Target dose (Gy)	No. females	Total eggs	Mean no. eggs per female (±SE)	Total eggs hatched	% eggs hatched (±SE)	Mean no. fertile eggs per female (±SE)
Early (1- to 3-d old)	0	90	3,700	35.6 (2.1)	1,438	40.5 (5.1)	13.8 (1.6)
	150	86	958	8.6 (2.0)	30	3.4 (1.1)	0.27 (0.11)
	200	74	510	4.6 (0.8)	14	3.0 (1.2)	0.13 (0.06)
	250	59	107	1.0 (0.5)	2	0.6 (0.6)	0.02 (0.02)
	300	53	94	0.9 (0.5)	0	0	0
	350	29	85	0.8 (0.3)	0	0	0
Late (8- to 10-d old)	0	399	35,144	81.4 (4.2)	13,722	36.7 (2.3)	31.8 (2.9)
	200	192	13,334	66.7 (5.3)	569	3.2 (0.8)	2.8 (0.8)
	250	388	21,026	46.9 (3.5)	1,078	5.6 (1.2)	2.4 (0.5)
	300	320	14,072	37.4 (2.6)	78	0.5 (0.1)	0.21 (0.05)
	350	148	4,962	24.8 (3.3)	3	0.03 (0.03)	0.02 (0.02)
	400	289	4,205	9.1 (1.4)	1	1.7 (1.7)	0.002 (0.002)

Late stage pupae were 8- to 10-d old and some members of the cohort were beginning to emerge as adults.

Table 6. Linear regressions on percentage egg hatch when *Epiphyas postvittana* early or late pupae were irradiated at 0, 200, 250, 300, 350, and 400 Gy

Stage	Obs.	Y-intercept (±SE)	Slope (±SE)	R^2	Predicted doses (±95% CL) to prevent egg hatch
Early pupae (1- to 3-d old)	5	32.3 ± 2.8	−0.12 ± 0.01	0.59	269.8 (242.5–305.2)
Late pupae (8- to 10-d old)	6	32.0 ± 1.2	−0.10 ± 0.004	0.61	327.1 (313.1–342.6)

Regression analysis used percentage egg hatch data from Table 5.

in oriental fruit moth is increased under low oxygen conditions as it is an many insects. These results suggest that 200 Gy is close to the threshold allowing some survivorship for oriental fruit moth. Typically, the highest dose applied during large-scale confirmatory testing is used as the minimum dose for the quarantine treatment (Follett and Griffin 2006); however, a radiation dose of 200 Gy was approved by USDA–APHIS for oriental fruit moth despite the upper limit measurement of 232 Gy during confirmatory testing. A safe and effective dose for oriental fruit moth and therefore a potential generic irradiation treatment for tortricid eggs and larvae with an adequate margin of safety might be 250 Gy. Establishing a generic dose for tortricid moths below 400 Gy could reduce treatment time for certain commodities thereby minimizing any negative effects irradiation treatment may have on commodity quality, reducing treatment costs, and increasing capacity for irradiation facilities (Follett and Neven 2006, Follett 2009).

The approved generic radiation treatment of 400 Gy for Insecta excludes the pupa and adult stages of Lepidoptera (USDA–APHIS 2006). Normally, fresh commodities exported using irradiation that may contain pupae or adults of a lepidopteran quarantine pest must be inspected and found free of the pest before export is permitted, and the presence of these stages of the pest could result in rejection. Development of a radiation dose to control the pupa and adult stages of a specific lepidopteran pest would prevent potential rejections. For pupae, radiation treatment between 150–250 Gy sterilized adults in koa seedworm, *Cryptophlebia illepida* (Butler) (Lepidoptera: Tortricidae) (Follett and Lower 2000), sweetpotato vine borer, *Omphisa anastomolsalis* (Guenee) (Lepidoptera: Pyralidae) (Follett 2006a), banana moth, *Opogona sacchari* (Bojer) (Lepidoptera: Tineidae) (Hollingsworth and Follett 2007), tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Arthur 2004), and Mexican leafroller, *Amorbia emigratella* Busck (Lepidoptera: Totricidae) (Follett 2008). Information on several other tortricid species is available from studies to develop sterilizing doses for sterile insect release (SIR) programs (e.g., Bloem et al. 2003, Suckling et al. 2007, Tate et al. 2007, IDIDAS 2007), and these studies indicate that radiation doses ≤200 Gy are sufficient to sterilize females.

Data presented here for *E. postvittana* suggest a radiation dose ≥400 Gy will be required to sterilize late-stage pupae. Applying a dose ≥400 Gy on a commercial scale may be difficult given dose uniformity ratios of 2–3 and the FDA limit of one kGy. Ultimately, the goal of a quarantine treatment is to prevent reproduction and propagation. Doses <400 Gy for *E. postvittana* pupae may be sufficient to prevent propagation and provide quarantine security if survivors show complete sex-bias, or if F_1 survivors die before developing to the adult stage. Male *E. postvittana* are known to be more radio tolerant than females (Soopaya et al. 2010, Jang et al. 2011). In our tests, larvae from fertile eggs were not reared to adult to determine their sex and ability to reproduce. Survivors at the higher radiation doses (300–400 Gy) may have been all males and therefore unable to propagate, or they may not have survived to adulthood to reproduce.

Individuals surviving radiation treatment also may have developed into sterile adults (North 1975). Inherited sterility has been used on a limited basis as an endpoint for quarantine treatments. Radiation treatment of reproductive adults at 150 Gy caused F_1 sterility in two diaspidid scale insects, *Pseudaulacaspis pentagona* (Targioni–Tozzetti) (white peach scale)

Table 7. Radiation doses reported to prevent adult emergence from treated tortricid larvae

Species	Common name	Dose (Gy)	No. tested	Reference
Clepsis spectrana (Treitschke)	Cyclamen tortrix	200	150	Wit and van de Vrie (1985)
Conopomorpha sinensis Bradley	Litchi stem end borer	250	2,532	Hu et al. (1999)
Cryptoplebia illepida (Butler)	Koa seedworm	250	11,256	Follett and Lower (2000)
Cryptophlebia ombrodelta (Lower)	Litchi fruit moth	250	11,256[a]	Follett and Lower (2000)
Ctenopseustis obliquana (Walker)	Brownheaded leafroller	160	100[b]	Lester and Barrington (2009)
Cydia pomonella (L.)	Codling moth	200	133,133	Mansour (2003)
Ecdytolopha aurantaiana Lima	Citrus fruit borer	200	100	Arthur (2004)
Epiphyas postvittana (Walker)	Light brown apple moth	150	37,947	Follett (this paper)
Grapholita molesta (Busck)	Oriental fruit moth	232	58,779	Hallman (2004)
Thaumatobia leucotreta (Meyrick)	False codling moth	150	1,940	Hofmeyr (unpublished)

[a] Follett and Lower (2000) showed that *C. illepida* was significantly more radiation tolerant than *C. ombrodelta* through direct comparison. Large-scale testing was performed with *C. illepida* only.

[b] Inferred from discussion of results and LD_{95} values; the actual no. of fifth instars treated and surviving at each dose tested was not reported.

and *Aspidiotus destructor* Signoret (so-called coconut scale) (Follett 2006b,c), and USDA–APHIS approved these radiation treatments for quarantine control (USDA–APHIS 2008). Inherited sterility is a realistic endpoint for quarantine control of *E. postvittana* pupae. Soopaya et al. (2011) showed that *E. postvittana* male pupae treated at a radiation dose of 250 Gy produced highly sterile (>99%) F_1 male offspring. Further research with a large number of individuals would be needed to identify a radiation treatment providing quarantine security for *E. postvittana* pupae using inherited sterility as an endpoint.

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